Isolation of genomic DNA from fin clips/embryos (96-well format)

Materials

- Proteinase K (PK): e.g. 20 mg/ml (200x) stock from Invitrogen
- Lysis buffer without PK: 10 mM Tris (pH 8.0), 100 mM NaCl, 10 mM EDTA, 0.4% SDS.

	10 ml
10 mM Tris-Cl, pH 8.0	**100 µl (1 M, pH 8.0)**
100 mM NaCl	**200 µl (5 M)**
10 mM EDTA	**200 µl (0.5 M, pH 8.0)**
0.4% SDS	**400 µl (10%)**
H_2O	**9 ml**

- 96-well plates: e.g. Corning 3344 (V-bottom wells retain DNA better)

Protocol

- Freshly prepare lysis buffer by adding PK to 100 µg/ml.
- Load 60 µl of lysis buffer into each well and then add the fin clip (small piece) or embryo.
- Seal the plate and incubate at 50ºC for 3 hr (The plate can "float" in a water bath).
- Resuspend the solution with a multichannel P200 to dissolve the tissue as much as you can (foaming is inevitable; filter tips are recommended).
- Add 60 µl of isopropanol (DO NOT resuspend; tissues tend to get stuck inside of tips), mix by gently swirling, and centrifuge at ~ 3,000 rpm/1,500x g for 5 min at 4ºC.
- A black pellet should be seen on the bottom of the well. Discard the supernatant by aspirating wells, clean tip for each well, avoid pellet.
- Add 100 µl of 70% ethanol, and repeat the 5-min centrifugation. Aspirate ethanol.
- Air-dry the pellet (SpeedVac is not recommended, as it tends to over-dry the pellet).
- Resuspend the pellet in 100 µl of water. It is normal to see residual tissues after extensive resuspension.
- Briefly spin down the plate and use the clear supernatant for restriction enzyme digestion or PCR.

Genotyping PCR (25 µl reaction)

The precipitated DNA from clipped fin was resuspended in 100 µl of water.

	µl	Final conc.
DNA prepared from fin-clip	1	
5 µM YFP (integration-specific primer)	2	0.4 µM
10 µM 3'LTR F primer	1	0.4 µM
10x PCR buffer w/o $MgCl_2$ (Invitrogen)	2.5	1x
50 mM $MgCl_2$ (Invitrogen)	0.75	1.5 mM
10 mM dNTP (each)	0.5	0.2 mM
10 µM MCT4F	0.5	0.2 µM
10 µM MCT4B	0.5	0.2 µM
Platinum Tag (Invitrogen)	0.1	
H_20	16.15	
Total	25	

PCR
94ºC, 2 min.
35 cycles of 94ºC, 30 sec- 57ºC, 30 sec- 72ºC, 30 sec.
72ºC, 5 min.
4ºC, forever

MCT4F/B amplify a genomic locus (monocarboxylate transporter 4), a 276-bp amplicon; it serves as the internal positive control to ensure the success of isolation of genomic DNA and PCR when genotyping outcrosses.

To design primers for genotyping retroviral integrations of inbred carriers in particular, we typically use the "standard" LTR primer pointing off the 3' end of the virus (3' LTR F primer). The gene specific primer 1 is designed to be just adjacent to the retroviral integration "downstream" of the 3' LTR (so knowing the

retroviral integration orientation is necessary). The resulting amplified fragment is always approximately the same size (≈500bp) for all integrations. Gene specific primer 2 is positioned on the other side of the retroviral integration. This primer will only amplify a product if there is no retroviral integration to interfere with the amplicon. It is optimal if this amplicon is significantly different in size than the integration specific amplification (≈300bp fragment is good). All three primers can be added simultaneously to the PCR reaction and all 3 fish types (wt, het, mut) can all be identified in a single amplification.

To determine retroviral orientation from Ensembl or UCSC browsers:

Ensembl

Ensembl lists a qStart and a Qend for each integration. If qStart>qEnd, then the retrovirus is on the + strand (left side of the flanking sequence at the integration site), i.e. 3'LTR F will read 5'-> 3' on the chromosome. If qStart<qEnd, then the retrovirus is on the – strand.

UCSC

UCSC has + and – strand listings for integrations. A + means that the retrovirus is in a

5'->3' orientation (left side of the flanking sequence at the integration site) and that the 3'LTR F primer will read 5' ->3' on the chromosome. A – means the opposite, that the 3' LTR F primer is oriented 3' ->5' on the chromosome.